Advances in Bioresearch
Adv. Biores., Vol 13 (5) September 2022: 45-53
©2022 Society of Education, India
Print ISSN 0976-4585; Online ISSN 2277-1573
Journal's URL:http://www.soeagra.com/abr.html
CODEN: ABRDC3
DOI: 10.15515/abr.0976-4585.13.5.4553

Advances in Bioresearch

ORIGINAL ARTICLE

Heat Stability and Mosquito Larvicidal activity of *Brahmi* Phytoconstituents

[1]Manali Vaidya , [1]Nikita Shet , [2]*Mythili K. Jeedigunta, [3]Janani Jacob, [4]Raghuvir R. Pissurlenkar, [5]Rahul Chodankar, [6]Ajit K. Mohanty, [7]Arun B. Joshi

[1]M.Pharm, Department of Pharmacognosy, Goa College of Pharmacy, Panaji, Goa, 403001.
[2]Assistant Professor, Dept. of Pharmacognosy, Goa College of Pharmacy, Panaji, Goa - 403001
[3]Junior Research Officer, Department of Phytochemistry, Natural Remedies Private Ltd. Benguluru, Karnataka, 560100.
[4] Associate Professor, Department of Pharmaceutical Chemistry, Goa College of Pharmacy, Panaji, Goa, 403001.
[5] Assistant Professor, Department of Pharmaceutical Chemistry, Goa College of Pharmacy, Panaji, Goa, 403001.
[6]Scientist B, ICMR- National Institute of Malaria Research, Panaji, Goa, 403001.
[7]Professor, Department of Pharmacognsoy, Goa College of Pharmacy, Panaji, Goa, 403001
* Corresponding Email: jsnrkmyth@gmail.com

ABSTRACT

Vector borne diseases, illnesses that occur due to the transmission of the parasites/pathogens are one of the main illnesses of human population. The efforts made in research so far could not completely over power these pathogens. More toil and travail are required in every possible direction to search for a good combat agent. Medicinal plants have undoubtedly been a good source of panacea for many ailments. In the present study one such medicinal plant, Brahmi, is examined concerning its larvicidal activity. The methanolic extract has been subjected to heat treatment for understanding any modifications in the major phytoconstituents; followed by a molecular modeling analysis of them with one of the major receptors (ecdysone) involved in the anti-larval activity. With the encouraging results obtained from the computational study a detailed bioassay was carried out using the extract on the three larvae Anopheles stephensi, Aedes aegypti and Culex quinquefasciatus. From the study it can be concluded that Brahmi can be used as a potent anti-larval agent in a formulation.

KEY WORDS: *Brahmi, Larvicidal, Molecular Modeling, Bioassay*

Received 10.07.2022 Revised 26.07.2021 Accepted 19.08.2022

How to cite this article:
M Vaidya , N Shet , M K. Jeedigunta, J Jacob, R R. Pissurlenkar, R Chodankar, A K. Mohanty, A B. Joshi. Heat Stability and Mosquito Larvicidal activity of *Brahmi* Phytoconstituents. Adv. Biores. Vol 13 [5] September 2022. 45-53

INTRODUCTION

The word *Brahmi* or *Brahman* represents the creative principle that lies realized in the whole world. It is the concept found in the *Vedas* and *Upanishads*. It is essentially related to all those properties which are related to the memory, learning and understanding of intellectual matter. *Brahmi* originates from *Brahma* which is the mind-born divine mother [1,2,3]. From this preamble, it can be easily perceived about the qualities of a plant which has been named *Brahmi*.

Two plants are frequently referred by this name *Brahmi* – *Bacopa monneiri* and *Centella asiatica*. However, the majority of the references correspond to the former and the latter is synonymously called *Mandukaparni* or *Gotu Kola*. Both the plants show significant differences morphologically and chemically which assists in easy identification but share an array of pharmacological activities. Morphological, majorly include the leaves of both the plants. Sessile and ovate-obovate leaves of *Brahmi* whereas petiolate and sub-reniform leaves of *Mandukaparni*, give a striking difference for identification. Chemically- though both contain the glycosides the aglycones differ. The glycosides of *Bacopa monneiri* are triterpenoid saponin with dammarane type (jujubogenin or pseudojujubogenin) and phenylethanoid

type of aglycones. The dammarane type of glycosides - Bacoside A {Bacoside A3, Bacopaside II, Bacopaside X and Bacopasaponin C} and Bacoside B {Bacopaside N1, Bacopaside N2, Bacopaside IV and Bacopaside V}[4-6]. These are considered as the major phyto-constituents responsible for the activity and are referred to as the biomarkers [7]. The phenylethanoid glycosides include the Monnieraside I-III and Plantainoside B. In addition to these *Bacopa* also contain Curcubitacins which are tetracyclic terpenes with steroidal structures which function as kairamones [8]. The phytoconstituents of *Centella asiatica* include glycosides with triterpene – Ursane-Oleanane type of aglycone, Madecassoside and Asiaticoside. In addition to these glycosides, it is also reported to contain polyacetylenes (Centellin, asiaticin and centelicin), flavonoids – quercetin, kaemferol and phytosterols – campsterol, sitosterol, stigmasterol[9].

Both plants have been reported to contain a wide range of pharmacological activities. However, the majority of the reports have been primarily about the cognitive enhancing effects with a specific mention about memory, concentration, and learning [10]. In addition to these, the other major activities reported are the cardiac and respiratory activity and in the treatment of certain neuropharmacological disorders like insomnia, insanity, depression, stress, epilepsy and psychosis [11]. They are also found to be possessing anti-inflammatory, antipyretic, analgesic, free radical scavenging, and lipid peroxidative activities [12]. However, *Bacopa* is considered to be more potent and safer than *Centella* and there is a degree of variation in the side effects as well.

In the present study *Brahmi (Bacopa monneiri)* has been considered. The methanolic extract is subjected to chromatography for the isolation of the biomarker Bacoside A, separated into its components and has been characterized with spectroscopic data in comparision with the standard literature. The extract has been subjected to heat treatment. The pattern observed after the heat treatment will assist in understanding the stability of Bacoside A especially during the process of soxhlet which is generally employed for extraction. Besides the established pharmacological activities, one of the effects which has not been analyzed in detail so far is the larvicidal activity of *Brahmi*. Based on the preliminary report about the larvicidal activity of the *aqueous* extract of *Brahmi*, detailed molecular docking studies were carried out using ecdysone receptor [13]. This is the key receptor involved in the hormonal regulation of various stages in the insect development (molting and metamorphosis of the epidermis and the nervous system)[14]. As the docking score indicated that the ligands of phenylethanoid glycosides and flavonoids of Brahmi have a greater docking score than the dammarane glycosides, the entire *methanolic* extract was subjected to bioassay against the 3rd instar larvae of *Anopheles stephensi*, *Culex quinquefasciatus* and *Aedes aegypti* mosquitoes.

MATERIAL AND METHODS

ISOLATION AND PURIFICATION:

The methanolic extract was subjected to column chromatography (Column:1) on silica gel (60-120 mesh) using a gradient elution of Pet. Ether: Ethyl acetate and ethyl acetate: Methanol with an increasing proportion of ethyl acetate and methanol respectively. A total of 41 fractions showing similar patterns were pooled. Fractions 15-18 eluted with ethyl acetate: Methanol (6:4) were further subjected to chromatography (Column:2) with reverse-phase silica (230-400 mesh) and gradient elution using chloroform: methanol: water (starting from chloroform 100% and changing it gradients and keeping methanol and water constant i.e., 7:1:0.1 to 1:1:0.1). 17 fractions were collected and concentrated on a rotary evaporator. Fractions 7-8 eluted using methanol: water (6:4) showed a similar pattern. This portion was subjected to reverse phase chromatography (Column:3) using silica (230-400 mesh). The column was subjected to gradient elution from 55% methanol: water to 100% methanol. From the 13 fractions obtained, fractions 2-5 showed similar peaks. These were pooled and further subjected to preparative HPLC using Kromasil C18 column and isocratic elution using 65% methanol:water. Two pure compounds BMM-01 (2g) and BMM-02 (3.2g) were obtained after rotary evaporation. The fraction 6 from the third column was further subjected to reverse phase chromatography (Column:4) using 55% methanol: water gradient till 100% methanol. From the fractions 9-10 a single compound BMM-03 (3.5g) was obtained. The fractions 7-9 from the third column were pooled and evaporated to give a pure compound of BMM-04 (2.4g). The individual isolated compounds have shown a positive reaction to qualitative tests like Libermann-Buchard test and Molisch test. The compounds were then determined for their melting point and other spectral characterization using IR, 1H and 13C NMR and Mass was performed and the data obtained were correlated with the standard.

HEAT TREATMENT:
About 50mg of *Bacopa monnieri* methanolic extract, Bacoside A, and the individual constituents were subjected to heat treatment at 110ºC in a hot air oven. The samples were collected after 2hrs. Subsequently, the samples were taken after 4hrs, 6hrs, 8hrs and 12hrs. The extract was targeted for Bacoside A and the individual constituents - Bacoside A3, Bacopaside II, Bacopaside X, and Bacopasaponin C.

MOLECULAR DOCKING STUDIES:
The docking studies were carried out by using Glide v7.8, Schrödinger Suite 2018 (Trial Edition) (Glide, Schrödinger, LLC, New York, NY, 2018.) on Intel hexacore processor-based linux workstation.[15-17]

PROTEIN AND LIGAND PREPARATION:
The X-ray crystal structure of ecdysone receptor (organism), PDB code 1R20 (was retrieved from Protein Data Bank[18,19]. The receptor crystal structure was prepared for docking using the Protein Preparation Wizard in the Schrodinger Suite 2018[20]. The bound crystal water was removed, hydrogen atoms were added and A chain was removed. The ionizable groups were optimized at a physiological pH of 7.0 followed by energy minimization using OPLS3 Force field to relax any crystal restrains on the structure till a convergence of 0.05 RMSD was met for the heavy atoms. The bacosides used in docking studies were prepared using Ligprep. (LigPrep, Schrödinger, LLC, New York, NY, 2018). The atom types and partial charges were assigned based on the OPLS3 force field. The molecules were ionized using Epik at pH 7.0 to generate possible tautomeric states; multiple conformations were identified while retaining the specific chirality.

DOCKING STUDIES:
A grid was generated in the active site to dock the bacosides based on the bound ligand. The extents of the inner and outer grid box were set to 15 Å and 20 Å along the x, y and z directions respectively, providing ample space for the generation of varied ligand confirmation at the ligand-binding pocket. The Vdw (Van der Waals) radius scaling was set to default 1.0 Å to soften the potential over the non-polar areas of the enzyme that lie within the grid extents and the partial atomic charges were set to 0.25 of the exact values. The receptor atoms outside the grid were not scaled. The hydroxyl group of the amino acid serine, threonine and tyrosine were set to rotate to increase the probability of hydrogen bond formation. Subsequent the bacosides were docked to the receptor to find the best poses which were scored by GlideScore SP.

DETERMINATION OF LARVICIDAL ACTIVITY:
The 3rd *instar* larvae of the mosquito species, *Anopheles stephensi, Aedes agyepti,* and *Culex quinquefasciatus* were used for the study. They were obtained from the insectary of National Institute of Malaria Research, Goa Theses immature were maintained at 27 ± 2°c, 70-80% relative humidity (RH).

SAMPLE PREPARATION:
A Hundred milligrams of methanolic extract of *Bacopa monnieri* was dissolved in Dimethyl Sulphoxide (DMSO) to obtain 100ppm, 200ppm, 300ppm, 500ppm, 800ppm and 1000ppm concentration.

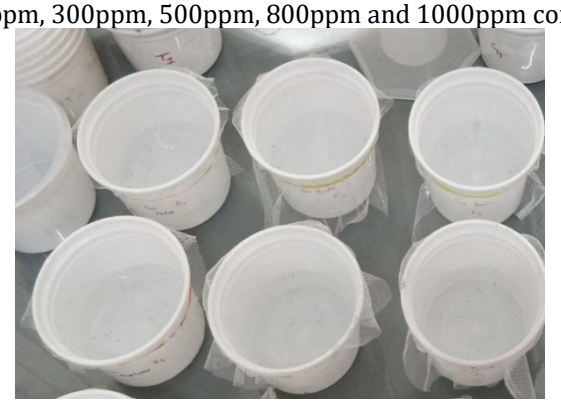

Figure no 1: Experimental set up for Larvicidal activity

The bowls used for the experiment were washed with sterile water and later on with spirit. The bowls were dried and then kept in the autoclave for 20 minutes. Different concentrations of the suspensions were fed to 25 healthy 3rd *instar* test larvae species in 500 ml plastic bowls containing 250ml of sterile distilled water. The percent mortality of the larvae was recorded after 24 and 48 hours of exposure by counting the living and dead larvae. All the tests were conducted under controlled temperature (28ºC± 2ºC) and each test was replicated at least thrice.[21] Activity of the extract against test mosquito larvae in terms of LC_{50} and LC_{90} was studied by analyzing dose mortality responses of individual strains by probit analysis using SPSS software.

If the mortality in control was more than 5%, Abbott's corrective formula was applied.
% Mortality = Number of dead larvae/total number of larvae*100
Corrected Mortality (%) = (% test mortality - % control mortality) /(100 - % control mortality)*100[21]

RESULTS AND DISCUSSION

CHARACTERIZATION:

Compound 1

Melting point: 247°C; IR (KBr) : 3445.01, 2943.50, 2870.20, 1678.14, 1454.39, 1375.30, 1289.47, 1079.22 cm^{-1}; LC-MS (negative) *m/z* 927.50 $(M-H)^-$; ^1H NMR (DMSO) and ^{13}C NMR data was compared with the standard. The compound was identified to be Bacoside A_3[22].

Compound 2

Melting point: 253°C; IR (KBr) : 3445.60, 2942.63, 1454.39, 1372.41, 1285.58, 1214.24, 1289.47, 1081.51 cm^{-1}; LC-MS (negative) *m/z* 927.33 $(M-H)^-$; ^1H NMR (DMSO) and ^{13}C NMR data was compared with the standard. The compound was identified as Bacopaside II[23].

Compound 3

Melting point: 230°C; IR (KBr): 3419.94, 2943.50, 2865.38, 1653.07, 1453.43, 1381.09, 1288.50, 1216.17, 1077.29 cm^{-1}; LC-MS (negative) *m/z* 897.25 $(M-H)^-$; ^1H NMR (DMSO) and ^{13}C NMR data was compared with the standard. The compound was identified as Jujubogenin isomer of Bacopasaponin C (Bacopaside X)[5].

Compound 4

Melting point: 291°C; IR (KBr): 3429.43, 2939.52, 1456.26, 1381.03, 1078.21 cm^{-1}; LC-MS (negative) *m/z* 897.67 $(M-H)^-$; ^1H NMR (DMSO) and ^{13}C NMR data was compared with the standard. The compound was identified as Bacopasaponin C[5].

HEAT TREATMENT:

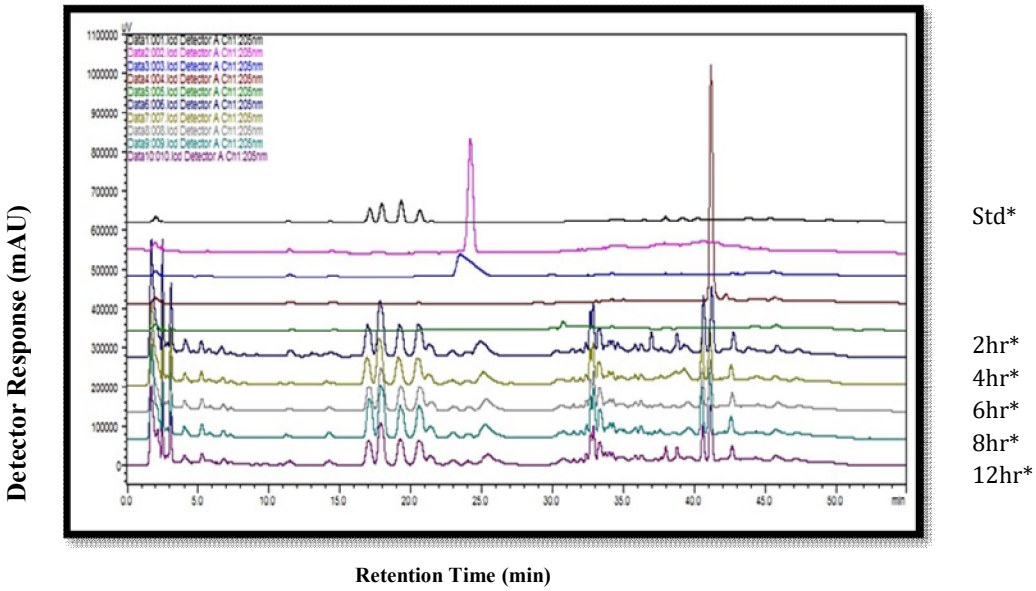

Figure No 2: HPLC of heat treated extract sample at 110 ºC

*-Detector response of the standard and the samples collected after respective time intervals

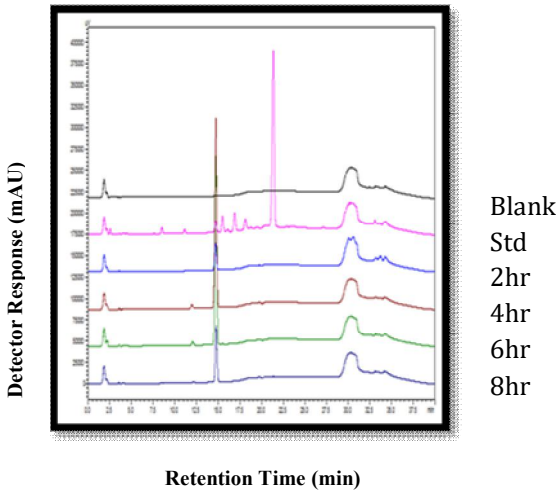

Figure No 3: (a) HPLC of heat treated Bacoside A_3
*-Detector response of the standard and the samples collected after respective time intervals

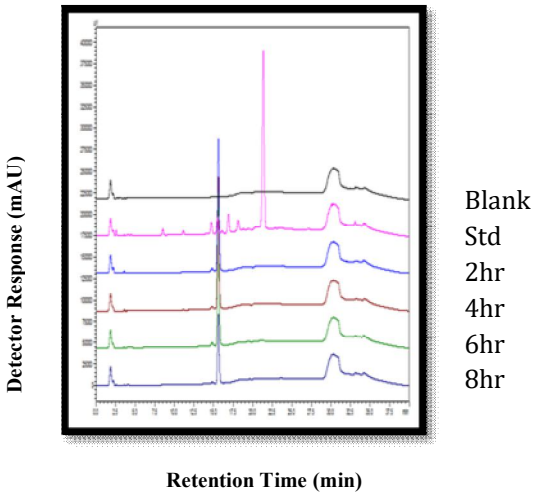

Figure No 3: (b) HPLC of heat treated Bacopaside II
*-Detector response of the standard and the samples collected after respective time intervals

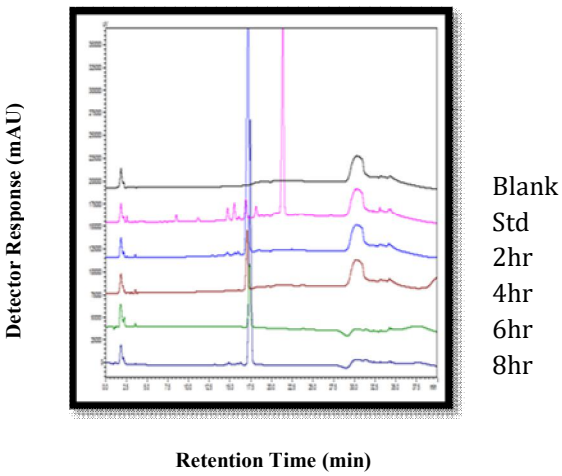

Figure No4:(a) HPLC of heat treated Bacopaside X
*-Detector response of the standard and the samples collected after respective time intervals

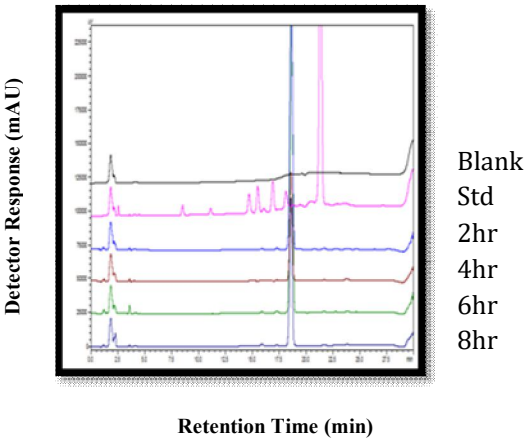

Figure No 4 (b) HPLC of heat treated Bacopasaponin C
*-Detector response of the standard and the samples collected after respective time intervals

The Figure No. 2 indicates the comparison of the standard of Bacoside A with that of the extract subjected to heat treatment at 110°C at an interval of 2hrs. The results showed no significant change in the pattern. Figure No. 3(a) and (b) as well as Figure No. 4(a) and (b), suggest no significant change in the individual components of the glycosides subjected to the heat treatment as well.

MOLECULAR DOCKING:

Ligands Docking score

1. Monnieraside C – GlideScore SP of -9.70
2. Plantainoside - Glide Score SP of -9.41
3. Monnieraside B - Glide Score SP of -9.00
4. Apigenin-7-glucoside - Glide Score SP of -8.36
5. Luteolin-7-glucoside - Glide Score SP of -7.96
6. Monnieraside A - Glide Score SP of -7.68
7. Jujubogenin isomer of Bacopasaponin C - Glide Score SP of -5.78

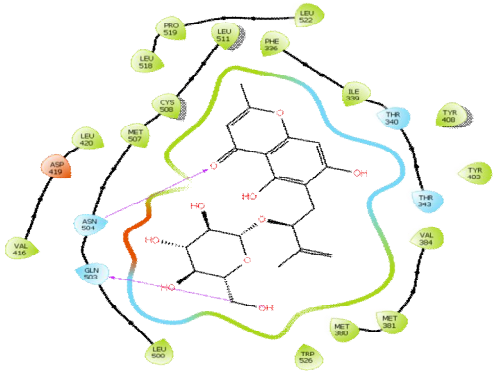

Figure No 5: Interaction of the ligand Monnieraside C with *Heliothis virescens* ecdysone

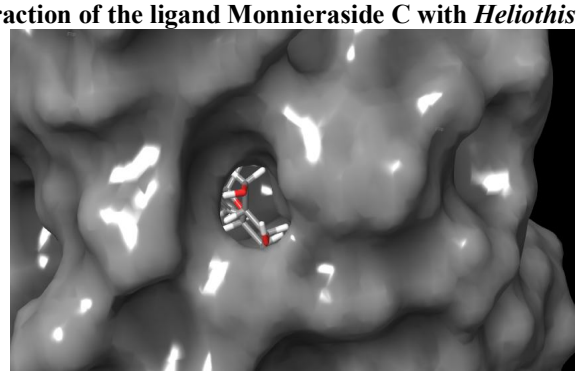

Figure No 6: Monnieraside C docked into the binding site of *Heliothis virescens* ecdysone receptor.

The comparison of docking score of various glycosides of the *Brahmi* with that of Ecdysone receptor suggested good docking of the constituents with Monnieraside C having the best Glide score. Fig No 5 and 6 show the three dimensional interaction between this ligand and the receptor. The binding interactions between all the ligands and the receptor has been shown in the Table No. 1.

Table No. 1: Binding interactions of the ligands to the *Heliothis virescens* ecdysone receptor

Serial no	Ligands	Interactions		
		Hydrogen bond	Hydrophobic	Polar
1	Monnieraside C (Fig no 5 and 6)	THR340, THR 343, GLN 503	ILE 339, LEU 522, PH 336, TRP 526, LEU 518, PRO 519, TYR 408, TYR 403, LEU 420, VAL 416, LEU 500, VAL 384, MET 381, MET 380, MET 507, CYC 508, LEU 511	THR 340, THR 343, ASN 504, GLN 503
2	Plantainoside	ASN 504, GLN 503,	LEU 522, PHE 336, LEU 511, PRO 519, LEU 518, CYS 508, MET 507, LEU 420, VAL 416, LEU 500, YRP 526, MET 380, MET 381, VAL 384, TYR 408, TYR 403, ILE 339	ASN 504, GLN 503, THR 340, THR 343
3	Monnieraside B	-	ILE 339, PHE 336, LEU 522, LEU 511, CYC 508, MET 507, LEU 518, TRP 526, TYR 408, TYR 403, VAL 384, MET 381, MET 380, LEU 420, ILE 417, VAL 416, TYR 415, MET 413, LEU 500	THR 340, ASN 596, THR 343, ASN 504, GLN503.
4	Apigenin-7-glucoside	GLN 503	TRP 526, TYR 408, ILE 339, TYR 403, MET 380, MET 381, LEU 420, LEU 500, VAL 416, TYR 415, MET 507	THR 343, THR 340, GLN 503, ASN 504, ASN 506, SER 510
5	Luteolin-7-glucoside	THR 343, TYR 408, TYR 415	TYR 403, ILE 339,LEU 511,PHE 336, LEU 518,LEU 522,TRP 526,TYR 408,MET 380,MET 381,LEU 420,VAL 416,TYR 415,MET 413,LEU 500, MET 507,CYC 508	THR 340,THR 343,ASN 506,ASN 504,GLN 503
6	Monnieraside A		TYR 403, MET 381, MET 380, LEU 522, ILE 339, PHE 336, TRP 526, LEU 511, TYR 408, CYC 508, MET 507, MET 413, TYR 415, VAL 416, LEU 500, LEU 420, MET 381, MET 380	THR 343, THR 340, ASN 504, GLN 503
7	Jujubogenin isomer of Bacopasaponin C	ASN506	ILE509, MET507, MET502, VAL 416, TYR 415	SER 510, ASN506, SER 506, ASN504, GLN 503, THR 499

LARVICIDAL ACTIVITY:

The larvicidal activity in terms of LC_{50} and LC_{90} values of the methanolic extract against laboratory reared 3rd instar larvae of *An. stephensi*, *Cx. quinquefasciatus* and *Aedes aegypti* are shown in table (2).

The methanolic extract was found more effective against *Culicine* followed by *Anopheline* and *Aedes* larvae after 24 and 48 hrs of exposure. Methanolic extract showed highest larval mortality against *Culex quinquefasciatus* with LC_{50} = 203ppm and LC_{90} = 682 ppm, followed by *An. stephensi* with LC_{50} = 406 ppm, LC_{90} = 795 ppm and *Aedes aegypti* with LC_{50} = 725 ppm, LC_{90} = 1194 ppm respectively at 24 hrs.

Plant based biocides have an advantage over synthetic pesticides because they are generally eco-friendly, readily biodegradable, target specific and less prone to the development of resistance in mosquitoes. The

present study has shown that the methanolic extracts of *Bacopa monnieri* cause extensive mortality in the 3rd *instar* larvae of all the three test mosquito species. This broad spectrum of activity is desirable in the integrated vector management (IVM) against many vector species simultaneously breeding in inter-specific associations.[24] This study suggests that methanolic extract of *Bacopa monnieri* could be developed as a potent herbal insecticide for the control of mosquito vectors.

Table No:2 Larvicidal activity of methanolic extract of *Bacopa monnieri* against third *instar* larvae of Mosquitoes

Mosquito species (n=25)	24 hours[a]		48 hours[a]	
	LC_{50}*	LC_{90}*	LC_{50}*	LC_{90}*
Anopheles stephensi	406.182 (323.831-509.476)	795.724 (634.395-998.079)	271.061 (211.616-347.205)	584.182 (456.068-748.285)
Aedes aegypti	725.798 (602.409-874.461)	1194.717 (991.609-1439.427)	300.027 (236.142-381.195)	617.589 (486.085-784.669)
Culex quinquefasciatus	203.546 (136.788-302.886)	682.915 (458.935-1016.207)	120.493 (86.676-167.503)	306.075 (220.175-425.490)

* - 95% Confidence limit (lower –upper)

[a] – time after which the readings were taken

n- the no.of third *instar* larvae of mosquitoes considered for the study

CONCLUSION

Brahmi (*Bacopa monnieri*) has been used in Ayurveda and other alternative systems of medicine for a wide array of pharmacological benefits. However, with the advent of modern technology and the scale of manufacture of the formulations, it is but imperative to understand the stability of the constituents during the processing. Also, one of the obligatory effects of the extract i.e larvicidal activity has been studied in detail taking the fulcrum of molecular docking studies for the confirmation. It can be concluded decisively that this significant herb is stable during various heat treatments it may be subjected to during extraction and has a perceivable larvicidal activity.

CONFLICT OF INTEREST: NIL

REFERENCES

1. Mukerjee DG, Dey CD. (1996) Clinical trial on Brahmi. J. Exp. Med. Sci. 10 Suppl 1: 5-11.
2. Kapoor LD. (2000). Hand Book of Ayurvedic Medicinal Plants. Boca Raton: CRC Press.
3. Garai S, Mahato SB, Ohtani K, Yamasaki K. (1996) Bacopasaponin D - a pseudojujubogenin glycoside from *Bacopa monnieri*. Phytochemistry. 43 Suppl 2:447-449.
4. Deepak M, Sangli GK, Arun PC, Amit A. (2005) Quantitative determination of the major saponin mixture bacoside A in *Bacopa monnieri* by HPLC. Phytochem. Anal. 16: 24-29.
5. Sivaramakrishna C, Rao CV, Trimurtulu G, Vanisree M, Subharaju GV. (2005) Triterpenoid glycosides from *Bacopa monnieri*. Phytochemistry. 66: 2719-2728.
6. Russo A, Borrelli F. (2005) *Bacopa monniera*, a reputed nootropic plant: an overview. Phytomedicine. 12 Suppl 4:305-317.
7. Deepak M, Amit A. (2004) The need for establishing identities of bacoside A and B, the putative major bioactive saponins of Indian medicinal plant *Bacopa monnieri*. Phytomedicine. 11: 264-268.
8. Pamita B, Neeraj K, Bikram S, Vijay KK. (2007) Cucurbitacins from *Bacopa monnieri*. Phytochemistry. 68:1248-1254.
9. Siddiqui BS, Aslam HS, Khan AS, Begum S. (2006) Chemical constituents of *Centella asiatica*. Journal of Asian Natural Product Research. 9 Suppl 4:407-414.
10. Nadkarni KM. (1988) The Indian Materia Medica. Bombay: Popular Prakashan.
11. Kishore K, Singh M. (2005) Effect of bacosides, an alcoholic extract of *Bacopa monniera* Linn. (brahmi), on experimental amnesia in mice. Indian J. Exp. Biol. 43 Suppl 7:640-645.
12. Anbarasi K, Vani G, Balakrishna K, Desai CS. (2005) Creatine kinase isoenzyme patterns upon exposure to cigaratte smoke: protective effect of Bacoside A. Vascul. Pharmacol. 42 Suppl 2:57-61.
13. Rathy MC, Sajith U, Harilal CC. (2015) Plant diversity for mosquito control: A preliminary study. International Journal of Mosquito Research. 2 Suppl 1: 29-33.
14. Riddiford LM, Cherbas P, Truman JW. (2000) Ecdysone receptor and their biological actions. Vitam. Horm. 60:1-73.

15. Friesner RA, Murphy RB, Repasky MP, Frye LL, Greenwood JR, Halgren T, et.al. (2006) Extra Precision Glide: Docking and Scoring Incorporating a Model of Hydrophobic Enclosure for Protein-Ligand Complexes. J. Med. Chem. 49 Suppl 21:6177–6196.
16. Halgren TA, Murphy RB, Friesner RA, Beard HS, Frye LL, Pollard WT, et.al. (2004) Glide: A New Approach for Rapid, Accurate Docking and Scoring. 2. Enrichment Factors in Database Screening. J. Med. Chem. 47 Suppl 7:1750–1759.
17. Friesner RA, Banks JL, Murphy RB, Halgren TA, Klicic JJ, Mainz DT, et.al. (2004) Glide: A New Approach for Rapid, Accurate Docking and Scoring. 1. Method and Assessment of Docking Accuracy. J. Med. Chem. 2004; 47 Suppl 7:1739–1749.
18. Billas IML, Iwema T, Garnier JM, Mitschler A, Rochel N, Moras D. (2003) Structural adaptability in the ligand binding pocket of the ecdysone receptor. Nature. 2003; 426:91-96.
19. Berman HM, Westbrook J, Feng Z, Gilliland G, Bhat TN, Weissig H, et.al. (2000) The Protein Data Bank. Nucleic Acids Research. 2000;28 Suppl 1:235-242.
20. Sastry GM, Adzhigirey M, Day T, Annabhimoju R, Sherman W. (2013) Protein and ligand preparation: Parameters, protocols, and influence on virtual screening enrichments. J. Comput. Aided. Mol. Des. 2013; 27 Suppl 3: 221-234.
21. Ajeet KM, Sandeep G, Kulvir D, Ashwani K. (2017) Variable Region of 16s rRNA is Essential for the Identification of Group 1 Mosquito- Pathogenic Strains of *Lysinibacillus*. Adv Biotech & Micro. 2017. 2 Suppl 2:555583.
22. Rastogi S, Pal R, Kulshreshtha DK. (1994) Bacoside A3-A triterpenoid saponin from *Bacopa monniera*. Phytochemistry. 1994; 36 Suppl 1:133-137.
23. Chakravarty AK, Sarkar T, Masuda K, Shiojima K, Nakane T, Kawahara N. (2001) Bacopaside I and II: two pseudojujubogenin glycosides from *Bacopa monniera*. Phytochemistry. 2001; 58 Suppl 4:553-556.
24. World Health Organization (WHO). Planning and Implementation. Handbook for Integrated Vector Management. Geneva, 2012. (WHO/HTM/NTD/VEM/2012.3): 32.